

Exclusive customized epigenetic antibodies

For many years, Diagenode has acquired a strong and reliable experience in the production of custom epigenetic antibodies, allowing us to become one of the most reliable partner in several EU projects in this field of studies. Following our success in optimizing and developing our epigenetic antibody production procedures we are able to offer this as a custom service to meet your specific research needs.

Our Exclusive Antibody Program has several unique features:

- **Flexibility:** you can provide us with your protein or with your own peptide or you can choose our custom peptide design/synthesis/coupling service.
- **Support:** our team of specialists will provide you with free peptide design and top-level advice and support along your immunisation program.
- **High-Quality products:** our Extended immunisation program will ensure you to get more Ab with higher titer and affinity than any other supplier. Moreover at the end of the program you will receive purified anti-serum.
- **Extension of partnership:** If you think that your antibody is proven to be of commercial interest, we will be pleased to consider the opportunity to collaborate.

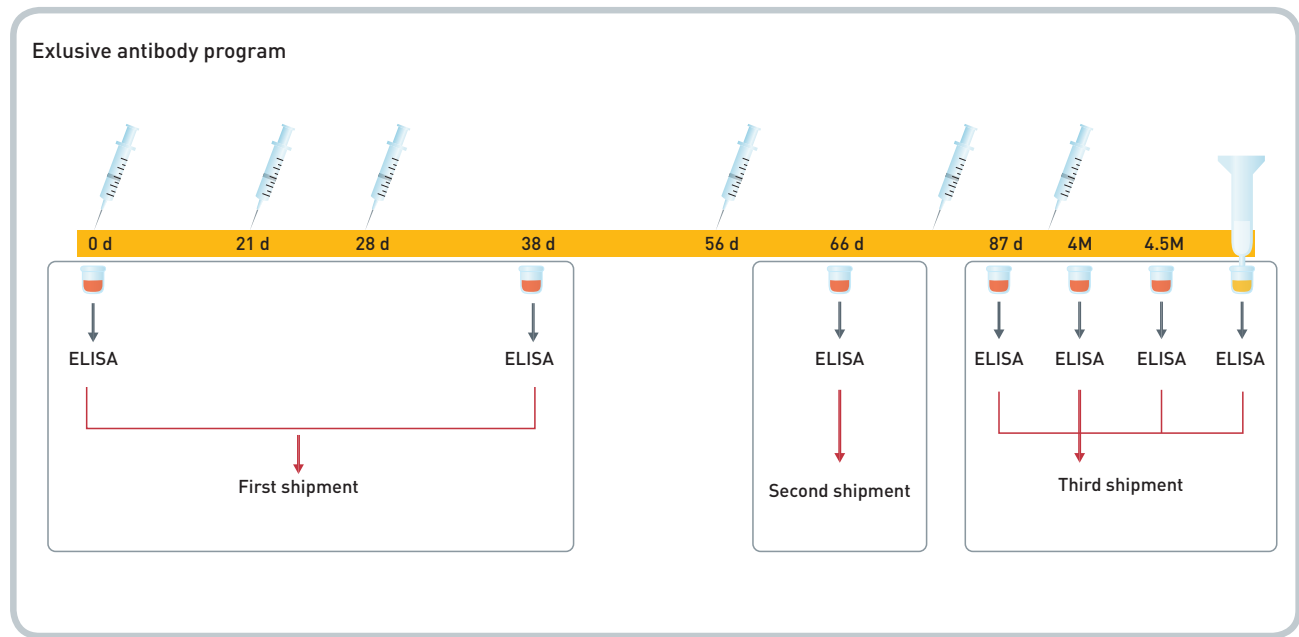
Our Diagenode antibody team works hard to develop the best peptide available and has access to specialised chemists for their synthesis. In addition to 'classical' peptide synthesis we have expertise with the following types of modifications: **Acetylation, Citrullination, Methylation, Phosphorylation, Sumoylation, ADP-ribosylation, Isopeptide bond...** and if you have a new idea or modification...our chemists are always ready for a new challenge!

Join our Exclusive Epigenetics User's Club

Through our involvement with EU consortiums, collaborations and inhouse experience we know that antibodies are the key to successful epigenetic experiments. Here at Diagenode we work exclusively with pockets of epigenetic researchers to develop and characterize the best epigenetic antibodies available. If you are currently researching or looking to start DNA methylation analysis or chromatin immunoprecipitation contact us today and join our Exclusive Epigenetics User's Network!

For more information please contact us (techsupport@diagenode.com).

Please find below an overview of the antibody production process.



1. Immunisation

Two rabbits are immunised for each custom antibody. Each rabbit is injected 6 times (see syringes on scheme).

For immunisation you can either provide us with a recombinant protein or with a synthetic peptide. We do peptide design and synthesis for you.

Peptide synthesis and coupling to KLH takes approximately one month.

2. Bleeds

Bleeds are taken on DAY 0 (PPI, negative control, ~1 ml), Day 38 (PP, ~1 ml), Day 66 (~20 ml), Day 87 (~20 ml), Month 4 (~20 ml) and Month 4.5 (SABC, Final bleed, ~ 50 ml).

All bleeds will be screened by an Enzyme Linked ImmunoAssay (ELISA) to determine the response of the rabbit to the immunisation. Note that we will only perform ELISA's in case of peptides were used for immunisation. These assays will only tell you if the antibody recognises the peptide(s), and not if it also recognises the target protein.

3. ELISA

We keep in house small aliquots of peptide(s) (~1 mg) & of all bleeds (~500 µl) to perform 3 ELISA's.

The 1st assay is done on Day 0 and Day 38 (the week of the 1st shipment), the 2nd on bleeds at Day 0, 38 & 66 (the week of the 2nd shipment) & the 3rd assay on all rabbits - all bleeds (minimum 11 days after Final bleeds).

4. First and second shipments

The first shipment contains crude sera at Day 0 and Day 38 bleeds and 5 mg of free peptide(s). The second shipment contains aliquots (~500 µl) of the crude sera at Day 66.

5. Optional purification

We keep in house another small aliquot of peptide(s) (~2.5 mg) to perform the purification of maximum of 10 ml of crude serum by affinity chromatography.

Based on your characterisation results (western blot, dot blot, IF, ChIP, ...) and our ELISA's, you can decide which bleed(s) you want to purify. As these are polyclonal antibodies, we recommend you to characterise all bleeds before.

At 4th ELISA, in which the purified antibody, the crude serum from which it was purified and the flow through are compared, will be performed as a quality control of the purification. As antibody production in terms of quantity depends on the rabbit's immune response, we can not predict or guarantee the amount of purified antibody.

6. Third shipment

The crude sera from all bleeds and all rabbits (~240 ml), the purified antibody and the remaining free peptide will be send to you in a third shipment.

7. Follow up

We will send you by e-mail at the beginning of your program a Time Table that summarizes the different steps of antibody production including expected dates for each step (please find an example of the Time Table here below).

Updated Time Table with shipments confirmation will be afterwards sent regularly. We will also send you ELISA results with titres calculation when available.

8. Overview

A custom antibody package includes:

- 1) Peptide design, peptide synthesis and peptide KLH coupling (~1 month)
- 2) Immunisation of 2 rabbits per target (4.5 months)
- 3) A total of ~240 ml of crude serum (~120 ml per rabbit)
- 4) Optional affinity antibody purification of maximum 10 ml from chosen crude serum
- 5) 4 ELISA QC
- 6) Three shipments free of charge

We will be happy to receive the results you obtain with the antibody.

Yours sincerely,

Geraldine Goens, Jan Hendrickx

R&D Epigenetics, Antibody Production, Diagenode sa.

TIME TABLE (EXAMPLE)

Date:

Your name and address:

Peptide 1	Sequence Peptide 1	2 rabbits
Peptide 2	Sequence Peptide 2	

		1st Shipment Date **			2nd Shipment Date **						
Ab#	Client	Peptide n°	Epitope name	Immunisation Contract	Rabbit n°	PPI	PP	LOT 001	LOT 002	LOT 003	LOT 004 (SABC)
#C	Your name	#Ref	Target	MARxxxx	Rabbit 1	Day 0	Day 38 ~1 ml	Day 66 ~20 ml	Day 87 ~20 ml	Month 4 ~20 ml	Final Bleed ~50ml
					Rabbit 2	Day 0	Day 38	Day 66	Day 87	Day 115	Day 129 (4.5 months)
						Day 0	Day 38	Day 66	Day 87	Day 115	Day 129 (4.5 months)

Comments:

1st Shipment :	**Minimum 11 days after Day 38	Crude sera at Days 0, Day 38 - all rabbits & 5 mg of free peptide
ELISA on Day 0 & Day 38 :	The week of the 1st shipment	
2nd Shipment :	**Minimum 11 days after Day 66	~ 500 µl of Crude sera at Day 66
ELISA Day 0, Day 38 & Day 66 :	The week of the 2nd shipment	
Optional Purification :	Depending on characterization done in your lab	
ELISA all bleeds :	Minimum 11 days after Final bleeds	
3rd Shipment :	Minimum 5 months after start & after the optional performed purification	Purified Antibody, all bleeds - all rabbits (~240 ml of crude sera) and remaining free peptide

Exclusive customized epigenetic antibodies [Cat. No. pAb-cust-045]		
Animal host	SPF Rabbit (2 per program)	
Antigen*	Anti-peptide immunisation program**	Polyclonal Anti-Peptide program - Peptide design and advice included - Synthesis of 20 mg of one 16-18 amino acids non-modified immunograde (> 70% purity) peptide*** - Coupling of 5mg to KLH protein carrier - ~ 5mg of free peptide sent with first bleed shipment - Remaining free peptide sent at program's end on customer's request.
	Anti-protein immunisation program	You can send us your protein lyophilised or in solution. Antigen purity must be > 80% (checked by SDS-PAGE, image should be provided along with the protein) to ensure a high-quality antibody production. A minimum protein quantity of 2mg is required per program. Please note that protein embedded in polyacrylamide gel slices are also accepted. For more information, please inquire.
Duration of the immunisation program	4.5 months****	
Number of injections	Total of 6 injections per animal (200µg antigen used per injection)	
Number of bleeds	6 (incl. Pre-Immune bleed - PPI)	0.5-1ml /rabbit (Pre-Immune Bleed - PPI): day 0 0.5 - 1 ml /rabbit: day 38 ~20 ml /rabbit: day 66 ~20 ml /rabbit: day 87 ~20 ml /rabbit: 4 months ~50 ml /rabbit (Final Bleed - SABC): 4.5 months
Purification	Differential affinity purification of 10ml serum from the best responding animal. Please note that ~2mg peptide are required per purification. Specificity of the purified antibodies is controlled by indirect ELISA testing.	
QC by ELISA	ELISA testing performed on all bleeds. Please note that ~1mg of peptides are required per program for ELISA QC.	
Shipping costs	All sera shipped on dry ice. Dry ice fees included (for countries outside Europe, please inquire)	The first shipment contains the Day 0 and Day 38 bleeds (~0.5ml per bleed) (incl.ELISA), the second shipment contains the Day 66 bleed (~20ml) (incl. ELISA). The entire remaining bleeds, the crude serum from the bleed(s) you have chosen and the purified antibody will be sent to you in a third shipment (incl. ELISA).
Optional	Additional QC	Additional QC using Dot Blot, Western blot, Immunofluorescence, ChIP, ChIP-Seq or ChIP-chip, are performed in client's lab with support of Diagenode team. In collaborative cases, when feasible, the Diagenode team may be able to carry out additional QC inhouse.
Price	2,500.00 €	

- * You can either provide us with your ready-to-use antigen or use our custom peptide design/synthesis/coupling service. In the first case the price is reduced of 600€.
- ** You might decide using two peptides for the immunisation. In this case, additional 600€ are charged (Price for 20 mg non modified peptide, including coupling of 5 mg to KLH, not including peptide modification).
- *** Price of peptide modification is not included. Please check our price list for price of the different modifications. Find below the list of modifications that are available.
- **** Excluding leadtime for peptide design/synthesis/coupling (only for anti-peptide immunisation program)

Available peptide modifications

Antibodies	Cat. No.	Format	€
Additional peptide synthesis (16-18aa, w/o modification)	pAb-cust-020	20 mg	600.00
Modification Ac-Lysine	pAb-cust-120	20 mg	150.00
Modification Monomethyl-Lysine	pAb-cust-220	20 mg	560.00
Modification Dimethyl-Lysine	pAb-cust-320	20 mg	300.00
Modification Trimethyl-Lysine	pAb-cust-420	20 mg	560.00
Modification Symetric Dimethyl-Arginine	pAb-cust-520	20 mg	900.00
Modification Phosphorylation	pAb-cust-620	20 mg	230.00
Modification Double Phosphorylation	pAb-cust-720	20 mg	460.00